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THE EFFECTS OF TEMPERATURE AND ACCLIMATION ON CRUSTACEAN NERVE-MUSCLE PHYSIOLOGY

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Abstract

Crustaceans, like most ectotherms, have body temperatures that are close to ambient. Although some animals live in constant warm conditions, most crustaceans live in environments with both short- and long-term temperature fluctuations. Rapid temperature changes generally produce changes in the properties of the nerve and muscle membranes. As a result increases in temperature generally cause a decrease in the effectiveness of neuromuscular transmission. This is offset by an increase in the amount of synaptic facilitation, an increase in axon firing frequencies, and in some motor axons the production of additional responses in the peripheral branches. Although these changes act to overcome temperature-induced decreases in muscle tension, little is known about how the intact animal utilizes these changes to produce coordinated movements at different temperatures.

Long-term changes in thermal conditions alter the properties of the motor nerves and the muscles. This results in a shift in the temperature range over which there is optimal neuromuscular performance towards the acclimation temperature.

Introduction

Many endotherms are well-insulated and are able to retain much of the heat produced during metabolism, so that body temperature is maintained within carefully defined limits. Ectotherms, by contrast, have a high thermal conductance so that any heat produced is quickly lost to the environment. As a result the internal temperature of many ectotherms is close to the ambient temperature. The problem faced by most ectotherms, therefore, is one of retaining a degree of functional integrity in a thermally fluctuating environment.

Certain ectotherms overcome this problem by living in an environment that provides near-constant thermal conditions. Hawaiian ghost crabs, for example, live in burrows and in the ocean at temperatures between 26 and 28°C, and their nocturnal habits ensure that they do not overheat in the sun. These crabs, therefore, utilize only minor behavioral modifications to maintain a near-constant body temperature. In the laboratory, however, exposure to cool temperatures results in these animals becoming sluggish and ultimately immobile with muscles that exhibit fibrillation, while warm temperatures cause jerky and erratic limb movements (Florey and Hoyle, 1976).

At the other extreme, certain ectotherms live in environments with temperatures that fluctuate dramatically. Animals that live in such environments must not only be able to withstand both short-term (daily) and long-term (seasonal) temperature changes, but must also remain functional over a wide thermal range. For example, the neuromuscular systems of shore crabs (*Pachygrapsus*) and crayfish (*Astacus*) can function over 15 and 24°C ranges, respectively (Harri and Florey, 1977; Stephens and Atwood,

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1982). It is apparent that in these animals certain compensatory mechanisms must be present that permit the animal to function over a wide temperature range. In crayfish, Kivivouri (1980) has shown that different neuronal pathways exhibit different resistances to short-term temperature changes. In general, complex reflexes are more sensitive to temperature change than simple reflexes, synaptic function is more sensitive than nerve conduction, and inhibitory junctions are more sensitive than excitatory (White, 1983). These results are similar to those described in goldfish (Prosser and Fahri, 1965; Prosser and Nagia, 1968; Friedlander *et al.*, 1976). In addition to withstanding rapid changes in temperature, ectotherms must also tolerate long-term thermal stress. In crayfish, for example, acclimation causes a shift in the lethal temperature (White, 1983) and modifies the temperature at which optimal activity takes place (Kivivouri, 1983). Moreover, when placed in an experimental regime in which animals can select their own environmental temperature, it has been shown that active animals prefer their ambient temperatures (Taylor, 1984, but see Crawshaw, 1983). Therefore, it is apparent certain mechanisms do exist that enable animals to modify their behavioral output in response to long-term changes in temperature.

The aim of the present paper is to describe the effects of short- and long-term temperature changes on the physiology of nerves and muscles, with special reference to crustaceans that normally live in a thermally fluctuating environment. Most of the work published to date has used neuromuscular preparations in walking limbs or claws. Since little work has been published that describes recordings from intact animals, most of the results outlined in this article were obtained using autotomized limb preparations which were bathed in a physiological saline, and whose temperature was carefully controlled and monitored.

Muscle Recordings

Resting potential

In crustacean muscle fibers the membrane potential increases with temperature. In crayfish, the membrane potential changes exhibit two linear components (Harri and Florey, 1977, 1979), whereas in crabs there are two logarithmic-linear components (Stephens and Atwood, 1982; Stephens, 1985). In both animals, the component observed at cooler temperatures has a steeper slope, and in some cases the change in membrane potential takes place faster than would be predicted by the Nernst equation. It has been suggested that the two components may be explained in terms of differential temperature effects on the membrane conductance to potassium and chloride, relative to sodium (Harri and Florey, 1977, 1979). Membrane potential recordings from crab and crayfish muscle fibers have revealed that the temperature at which the two components intersect can be changed by altering the acclimation temperature (Harri and Florey, 1979; Stephens and Atwood, 1982). Acclimation to different temperatures causes changes in the fluidity and saturation of lipids in membrane of goldfish (Roots and Johnston, 1968; Driedzic *et al.*, 1977; Matheson *et al.*, 1980), and crustaceans (Chapelle, 1977, 1978; Chapelle *et al.*, 1979). Thus, acclimation could produce certain fundamental changes in membrane composition, which may differentially influence the operation of channels for potassium, sodium, and chloride ions, as well as those concerned with leakage. Such a differential effect on the relative permeabilities of a membrane to different ions has been demonstrated in barnacles (Dipolo and Latorre, 1972; Fischbarg, 1972). Recently, it has been shown that the membrane potential can hyperpolarize beyond the potassium potential at high temperatures (White, 1983). This has been explained in terms of temperature-induced changes in the properties of the sodium-potassium pump, producing a change in the distribution of these ions

across the membrane (Florey and Hoyle, 1976). Evidence for this comes from the observation that the rate of spontaneous motor neuron firing can be changed by temperature-induced changes in the sodium pump (Arechiga and Cerbon, 1981).

Irrespective of the mechanism that produces the two-phase relationship between temperature and membrane potential, one interesting phenomenon is that cold temperatures cause the muscle membrane to become depolarized above the excitation-contraction threshold, which is independent of temperature (Dudel and Ruedel, 1968), and creates passive tension in the muscle (Harri and Florey, 1977; Fischer and Florey, 1981). The amount of tension produced is dependent upon the amount of depolarization (Orkand, 1962) and can be released by direct action of the inhibitor on the muscle fiber. This suggests that post-synaptic inhibition may play a significant role in the behavior of an animal at cold temperatures.

Excitatory junctional potentials

In many crustacean muscle fibers the amplitude and the time course of the nerve evoked excitatory junction potential (ejp) (Fig. 1A, B) and the tension produced in the muscle decline with temperature (Harri and Florey, 1977; Stephens and Atwood,

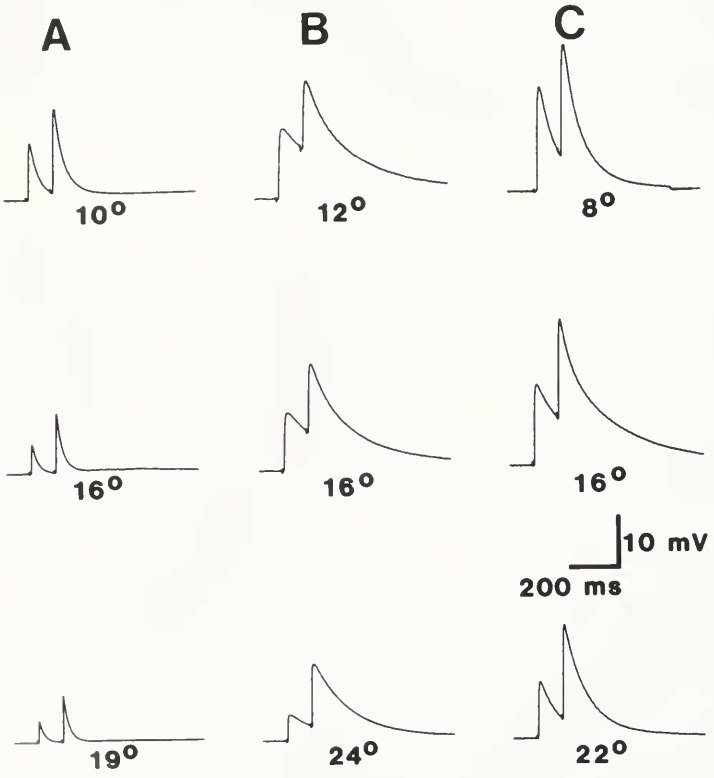

FIGURE 1. Examples of the effect of temperature on ejp's recorded from three different types of fibers in the stretcher muscle in a walking leg of *Pachygrapsus crassipes*. The pen recordings show changes in amplitude, time course, and facilitation, with a 100 ms interpulse interval (from Stephens and Atwood, 1983).

1983; White, 1983). The change in ejp time course can be fully accounted for by changes in the membrane conductance (Fischer and Florey, 1981). The decline in muscle tension with temperature is considered to be due to three factors: (1) depolarization of the membrane potential away from the temperature independent excitation-contraction threshold; (2) decline in ejp amplitude resulting in a smaller depolarization of the membrane following each impulse; and (3) decline in ejp time course which results in less summation of successive ejp's. These three factors result in a decline in neuromuscular efficacy with temperature.

In some muscle fibers ejp amplitude does not simply decline with temperature, but shows an initial increase followed by a decline (Fig. 1C). Examples of axons with this feature include the slow closer excitor to the crayfish closer muscle (Harri and Florey, 1977), the frog sartorius neuromuscular junction (Jensen, 1976; White, 1976), the rat phrenic-diaphragm (Hubbard *et al.*, 1971; Ward *et al.*, 1972), and the stretcher excitor and the slow bender excitor in walking legs of the Californian shore crab (*Pachygrapsus crassipes*) (Stephens and Atwood, 1982; Stephens, 1985). In certain stretcher muscle fibers the ejp amplitude and time constant have maximum values at the acclimation temperature (Stephens and Atwood, 1982). It has been suggested that the decline in ejp amplitude at cold temperatures may be due to conduction block in certain nerve branches (Hatt and Smith, 1975; Lang and Govind, 1977), or to temperature-induced changes in quantal content (White, 1983) perhaps produced by altering the amount of calcium entering the terminal (Charlton and Atwood, 1979).

In the crab stretcher muscle the degree of ejp facilitation, measured by comparing the relative amplitudes of pairs of responses evoked at different time intervals, is dependent upon temperature. Around the acclimation temperature, when the amplitude and time course of the ejp are at a maximum, facilitation (at frequencies > 10 Hz) is at a minimum (Stephens and Atwood, 1982). Changes in temperature cause a decline in ejp amplitude and time constant, but an increase in facilitation. As a result, the amount of tension produced by the muscle in response to short trains of excitatory axon spikes remains approximately constant within an 8°C range around the acclimation temperature. In this way the neuromuscular apparatus of the crab compensates for small temperature fluctuations.

It is interesting that the slow excitor to the bender muscle produces ejp's with some features that are different from those recorded from the antagonistic stretcher muscle. Bender muscle ejp's have maximum amplitudes and exhibit minimum facilitation around the acclimation temperature, as in the stretcher. However, increases in temperature result in a decline in the ejp time course, which can be fully accounted for by changes in the membrane input resistance (Stephens, 1985); an inverse relationship between membrane resistance and temperature has been reported in other crustacean muscles (Fatt and Katz, 1953; Fischer and Florey, 1981; White, 1983). Interestingly, the amount of tension produced by the bender muscle does not exhibit a temperature range over which it remains constant but also simply declines with temperature. These data suggest that tension is more closely linked to ejp time course than to amplitude. How the differential production of tension in antagonistic muscles affects the behavior of the crab at different temperatures has not been defined.

The stretcher muscle is innervated by a single excitatory motor neuron (Wiersma and Ripley, 1952), which has a diverse array of synaptic types (Fig. 1; Atwood, 1967). It is possible, therefore, to record from some fibers with synapses that produced small ejp's and good facilitation, and from other fibers with large amplitude ejp's that exhibit defacilitation or depression (Atwood and Bittner, 1971). The amount of facilitation or depression can be measured by producing pairs of ejp's at different intervals and

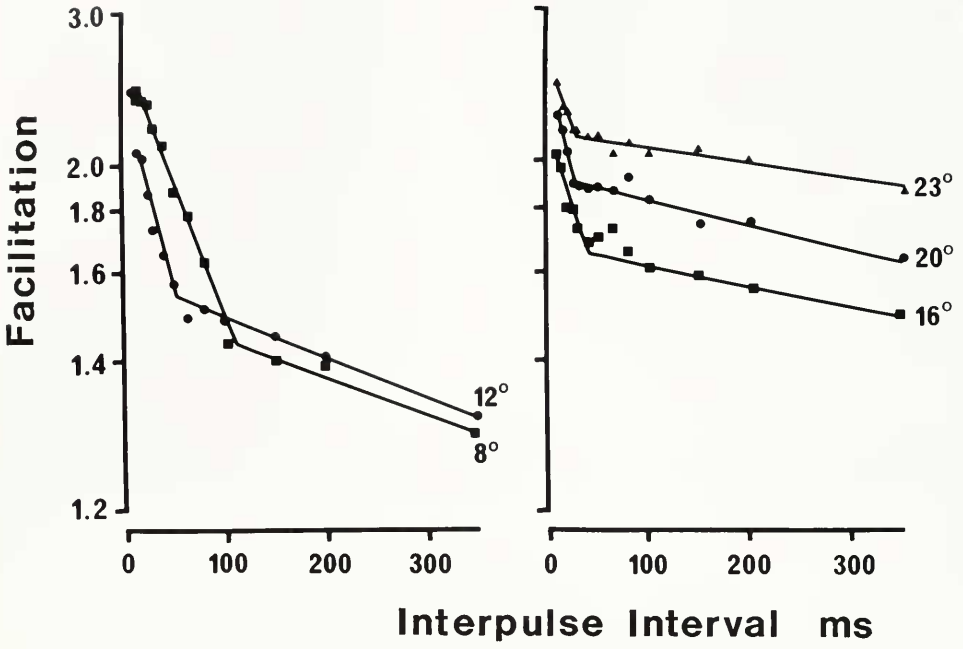

FIGURE 2. The effect of temperature on a highly facilitating muscle fiber in the stretcher muscle in a walking leg of *Pachygrapsus crassipes*. Facilitation was measured by producing pairs of ejp's at different intervals and comparing the amplitude of the second with the first response (from Stephens and Atwood, 1983).

comparing the amplitude of the second response with the first. When the measurements are corrected for non-linear summation (Martin, 1955), the values are very similar to those obtained from extracellular recordings of synaptic currents from the same fiber (Stephens and Atwood, 1983). In fibers with small amplitude ejp's, the amount of facilitation decays as the time interval between the ejp's is increased (Fig. 2). This decay in the amount of facilitation can be divided into two logarithmic-linear components (F_1 and F_2), which have been explained in terms of a decline in the probability of transmitter release following stimulation, possibly related to two different processes for the removal of calcium (Kita *et al.*, 1980). Increasing temperature causes the slope of F_1 to increase, the slope of F_2 to first increase and then decrease, and the time interval at which the two components intersected to decrease (Fig. 2). Thus, in these types of fibers, as temperature is increased the F_2 component becomes more dominant and the amount of facilitation shows an overall increase.

In the case of the large amplitude ejp's, temperature changes cause the facilitation profiles to change dramatically (Fig. 3). When ejp's are evoked at short time intervals, the amount of defacilitation decreases with increased temperature. When ejp's are elicited at longer time intervals, temperature increases result in defacilitation being replaced by facilitation. Extracellular recordings of focal synaptic currents from the muscle surface have revealed that this effect is not produced by temperature-induced changes in the efficacy of a heterogeneous population of synapses on single muscle fibers. Instead, synapses that produce large amplitude, de-facilitating ejp's at low temperatures change with increased temperature to produce small amplitude, facilitating

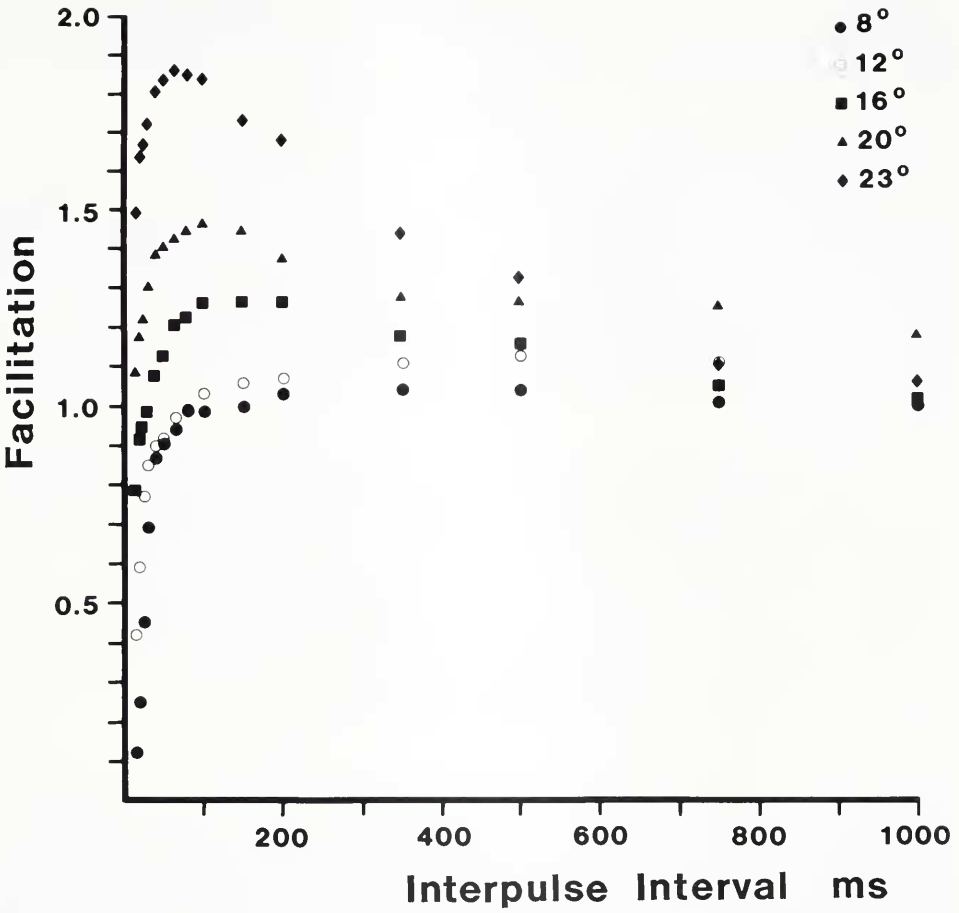

FIGURE 3. The effect of temperature on synaptic depression recorded from a single fiber in the stretcher muscle in a walking leg of *Pachygrapsus crassipes*. The graph shows the amount of facilitation at different temperatures. Facilitation was measured by producing pairs of ejp's at different intervals and comparing the amplitude of the second with the first response. Note that values > 1 indicate facilitation, while values < 1 indicate depression (from Stephens and Atwood, 1983).

ejp's. One possible mechanism to explain this observation is simply that increased temperature reduces spike duration. This has been shown in the axon in the merus (Stephens *et al.*, 1983) and in the terminals (Dixon and Atwood, pers. comm.). This temperature-induced decrease in spike duration would not only result in the release of less transmitter at the terminal and produce a small ejp, but would also leave more of the transmitter store available for release by the second action potential. However, broadening the spike with 3-aminopyridine (a potassium channel blocker) has little effect on the amount of facilitation observed at high temperature (Stephens and Atwood, 1983).

It is interesting that long-term facilitation, in which the amount of transmitter released at the synaptic terminals increases during maintained nerve activity, is also temperature sensitive (Jacobs and Atwood, 1981a). This type of facilitation occurs at

its optimum at or below the acclimation temperature, suggesting that long-term facilitation may be one mechanism in which neuromuscular performance may be enhanced at low temperatures.

Axon Recordings

Resting potential

As in muscle fibers, the axonal membrane potential declines with temperature (Dalton and Hendrix, 1962; Stephens, 1985). In the slow excitatory axon (E2) to the crab bender muscle there are two logarithmic-linear components, the component with the steeper slope being observed at cooler temperatures. As found in the muscle, the two components intersect at a temperature around acclimation (Stephens, 1985). The similar relationships between membrane potential and temperature for the motor axon and the muscle fibers indicate similar thermal effects on both membranes.

Action potential

In the crab motor axon (E2) to the bender muscle the amplitude and the time course of the action potential decline exponentially with temperature with Q_{10} values of 1.2 and 1.97, respectively (Stephens, 1985). The input resistance also declines with temperature (see also Colton and Freeman, 1975) with a Q_{10} of 2.0. This value is not significantly different from that obtained for the time course of the spike.

The axonal spike is followed by a hyperpolarizing after-potential in the lobster giant axon (Dalton and Hendrix, 1962) and a depolarizing after-potential in the E2 axon to the crab bender muscle (Stephens *et al.*, 1983). In both of these examples, the magnitude of the after-potential increases with temperature.

Peripheral generation of action potentials

In the Californian shore crab (*P. crassipes*) increasing the temperature above a critical threshold causes a single action potential in the stretcher excitor to produce more than one action potential and ejp (Fig. 4A; Stephens and Atwood, 1981). This effect can be reversed by cooling the preparation below the threshold temperature. The temperature threshold for this effect can be altered by acclimation (Stephens and Atwood, 1981) and by bath applications of low levels of ethanol (Stephens and Lazarus, 1981; Lazarus *et al.*, 1982). During a burst, the number of additional nerve spikes increases with temperature and is always matched with a concomitant number of ejp's (Fig. 4B–D—panel E). Moreover, the additional responses are produced at such a frequency that the ejp's undergo summation and facilitation and result in an increase in muscle tension (Stephens, 1985).

Extracellular recordings from two locations along the axon reveal that the additional spikes are generated in the periphery and travel antidromically down the axon (Stephens and Atwood, 1981). Similar observations have been made for the specific inhibitor to the stretcher muscle (Fig. 4B–D—panel SI) and the slow excitor (E2) to the bender muscle, the stretcher excitor of the green crab *Carcinus maenas* (Stephens, unpub. obs.), and in motor neurons in *Sh Drosophila* mutants (Ganetzky and Wu, 1982). Furthermore, observations that are consistent with this phenomenon have been recorded extracellularly from the bender muscle in intact shore crabs (Lazarus *et al.*, 1982).

The additional spikes that are generated in the periphery of the stretcher excitor axon can be modulated by activity in the specific inhibitor (Fig. 4). In this case, there is always a matching between the number of excitatory axon spikes and the number of ejp's (Fig. 4B–D—panel E + SI), suggesting that the point of spike modulation is

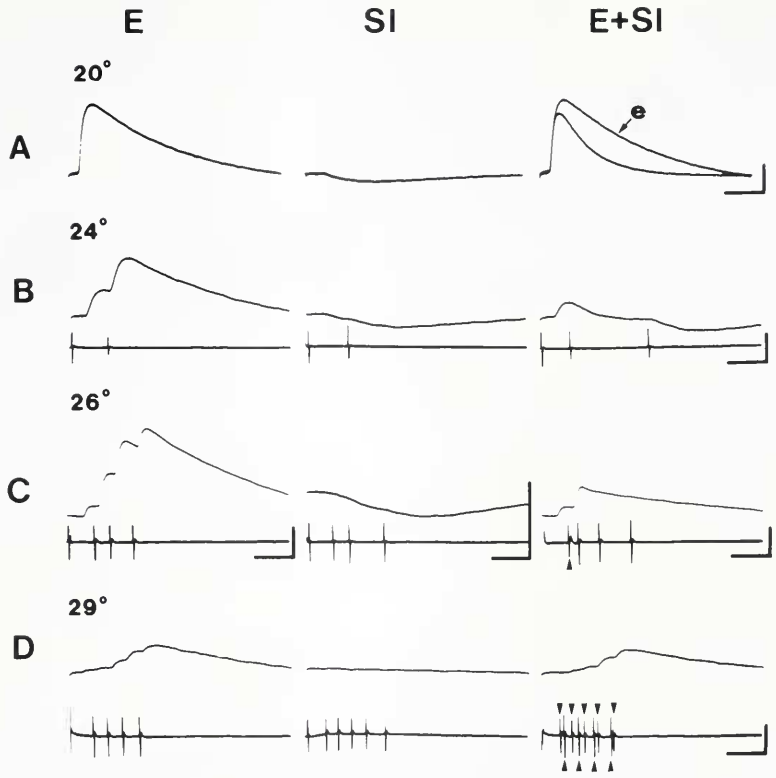

FIGURE 4. Junctional potentials and nerve spikes recorded following stimulation of the excitor (E) and the specific inhibitor (SI) axons to the stretcher muscle in an autotomized walking leg of *Pachygrapsus crassipes*. A. Recordings at 20°C showing the ejp (e) superimposed upon the response when both axons were stimulated simultaneously. B. Stimulation of either axon provoked junctional potentials and two nerve spikes. Synchronous stimulation of both axons abolished the second ejp and the second E axon spike. C. Stimulation of either axon provoked four junctional potentials and four axon spikes. Stimulation of SI after E abolished 2 ejp's and 2 E axon spikes. Note the E and SI spikes can be differentiated on the basis of contrasting amplitudes; the arrow points to the single antidromic E axon spike. D. At 29°C the SI spike-ijp coupling had broken down. Synchronous stimulation of both axons did not abolish the ejp's, but resulted in four peripherally generated E spikes (arrows below trace) and five peripherally generated SI spikes (arrows above trace). Calibration 5 mV and 10 ms (from Stephens and Atwood, 1981).

very close to the point where the additional spikes are generated. Moreover, the additional spikes can be abolished by bathing the preparation in gamma-aminobutyric acid, the inhibitory synaptic transmitter (Otsuka *et al.*, 1966), and the modulating effect of the inhibitor can be abolished by bath application of picrotoxin, an antagonist to inhibitory transmission (Takeuchi and Takeuchi, 1969). These data suggest that the points of spike generation and modulation are in the area of the axo-axonal synapses between the specific inhibitor and the excitor, possibly at excitatory axon branch points or "bottlenecks" (Jahromi and Atwood, 1974).

The phenomenon of peripheral spike generation has been recorded from the single excitor and the specific inhibitor to the crab stretcher muscle (Fig. 4) and from the slow (E2) excitor to the antagonistic bender muscle (Figs. 5E and 6C, D). It is interesting that action potentials recorded from the three axons consist of a spike followed by a depolarizing after-potential (Fig. 5B). Other axons that have a hyperpolarizing after-

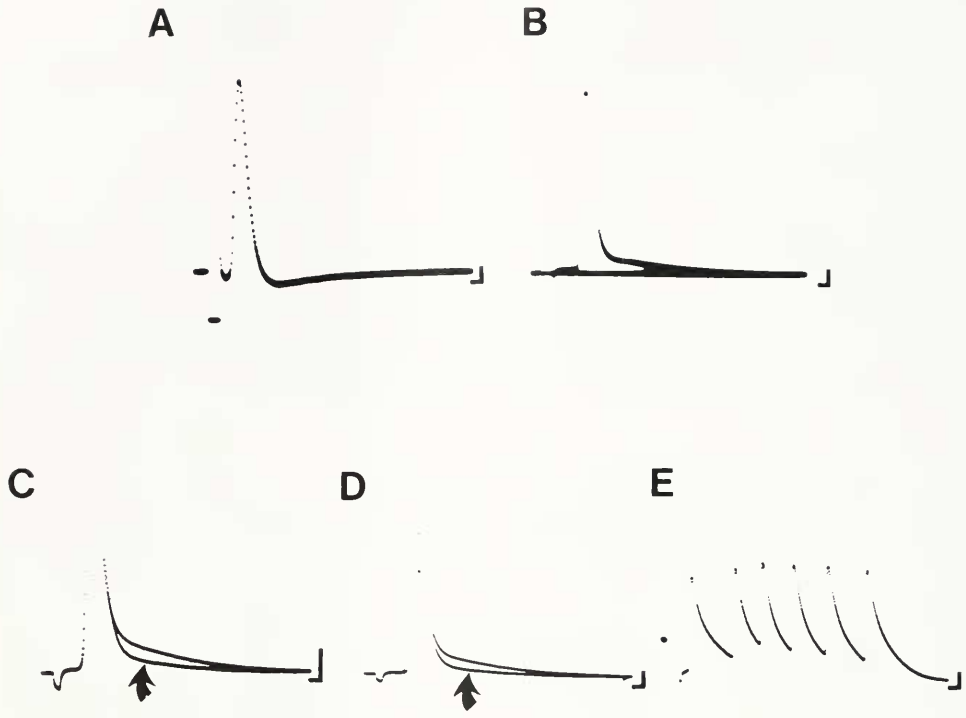

FIGURE 5. The effect of temperature and ethanol on the action potential recorded from excitatory axons to the bender muscle in an autotomized walking leg of *Pachygrapsus crassipes*. The action potentials recorded from E1 (A) and E2 (B) axons show hyperpolarizing and depolarizing after-potentials, respectively. Bathing preparations in saline containing 2% ethanol (C) or heating to a warm (26°C) temperatures (D) provoked an increase in the amplitude of the depolarizing after-potential in the E2 axon; traces are superimposed on an action potential recorded in normal saline at 14°C (arrow). Warming to 30°C resulted in a single axon shock provoking a train of E2 action potentials. Calibration 10 mV and 1 ms (from Stephens *et al.*, 1983).

potential, for example the fast excitatory (E1) axon to the bender muscle (Fig. 5A), do not exhibit peripheral spike generation. Increasing the temperature or bath applications of ethanol, both of which lower the temperature threshold for peripheral spike generation (Stephens and Lazarus, 1981), increases the size of the depolarizing after-potential (Fig. 5C, D). Further, elevating the temperature decreases the time course of the refractory period, so that at warm temperatures the elevated membrane potential produced by the depolarizing after-potential creates a period of decreased threshold (Stephens *et al.*, 1983). It has been suggested that the increased membrane resistance encountered where the axon becomes narrow, at branch points and "bottlenecks," increases the size of the depolarizing after-potential (Stephens *et al.*, 1983). If this is the case, immediately following the refractory period the increased membrane depolarization will be above threshold and additional action potentials will be generated at the periphery. Whether temperature influences the threshold for action potential production has not been established in crab axons.

If this hypothesis is correct, it is apparent that the generation of additional action potentials in the peripheral branches of axons is closely linked to the temperature-

sensitive depolarizing after-potential. In crayfish and *Aplysia* neurons, the depolarizing after-potential has been explained in terms of an increase in calcium conductance (Yamagishi and Grundfest, 1971; Lewis, 1984). This is particularly interesting since calcium and sodium are also considered to be involved in the spike portion of the action potential (Yamagishi and Grundfest, 1971; Kawai and Niwa, 1980). Therefore, it seems that increased temperature affects the sodium and calcium currents to decrease the size of the spike, but has an influence on other calcium currents to increase the magnitude of the depolarizing after-potential (Stephens *et al.*, 1983). The mechanism underlying this apparent contradiction remains to be understood.

The generation of additional spikes in the peripheral branches of certain axons is one way in which neural firing patterns can be altered by temperature. Since the pattern of motor impulses influences the amount of tension produced by the muscle (Wiersma and Adams, 1949), this dramatic change in axon firing at high temperatures may compensate for the decreasing effectiveness of the neuromuscular junction and may be one mechanism whereby ectotherms extend their thermal range. However, if animals lack the ability to coordinate this additional activity the result will be a series of erratic and jerky movements, as described in Hawaiian ghost crabs at high temperatures (Florey and Hoyle, 1976). The temperature threshold for peripheral spike generation is not the same for the stretcher excitor and the E2 bender excitor (Fig. 6C). The number of ejp's and the frequency at which they are produced at a particular temperature are not always the same (Fig. 6D). Also, the additional spikes in the stretcher excitor can be modulated (Fig. 4B–D—panel E + SI) while those in the E2 axon can not (Lazarus *et al.*, 1982). As a result of these factors, it seems likely that this differential effect of temperature on the motor supplies to antagonistic muscles will create significant coordination problems in the intact animal. Additional problems will be encountered when two axons are involved, one that shows peripheral spike generation and the other that does not, as in the excitatory motor supply to the bender muscle (Stephens *et al.*, 1983). The extent of these coordination problems in the intact animal has not been established.

Axon firing patterns can be altered by temperature. This has been shown in sensory neurons (Hatt, 1983), interneurons (*e.g.*, Langley, 1979; Prior and Grega, 1982; Nelson and Prosser, 1981), and motor neurons (Arechiga and Cerbon, 1981; and see Fischer and Florey, 1981). Temperature-induced changes in the conduction velocity and firing patterns of neurons may alter the phases between oscillator neurons and feedback relationships and thereby change particular behavior patterns. This has been reported in the Hawaiian ghost crab, where running speed is closely correlated with temperature (Florey and Hoyle, 1976). It is apparent that certain ectotherms can compensate for temperature-induced changes in the effectiveness of nerve and muscle function with the result that they remain functional throughout a wide temperature range, however the mechanisms by which this is achieved are poorly understood.

Considerations for the Future

Based on the above data, one outstanding problem involves attempting to explain the mechanism underlying the two-phase relationship between temperature and membrane potential. It has been suggested that temperature may influence the sodium-potassium pump and/or have a differential effect on ion channels in the membrane. Answers to this problem could be obtained using techniques such as ion-selective electrodes, to examine whether the levels of potassium or chloride ions inside nerve and muscles are altered by changes in temperature. The use of voltage clamp techniques

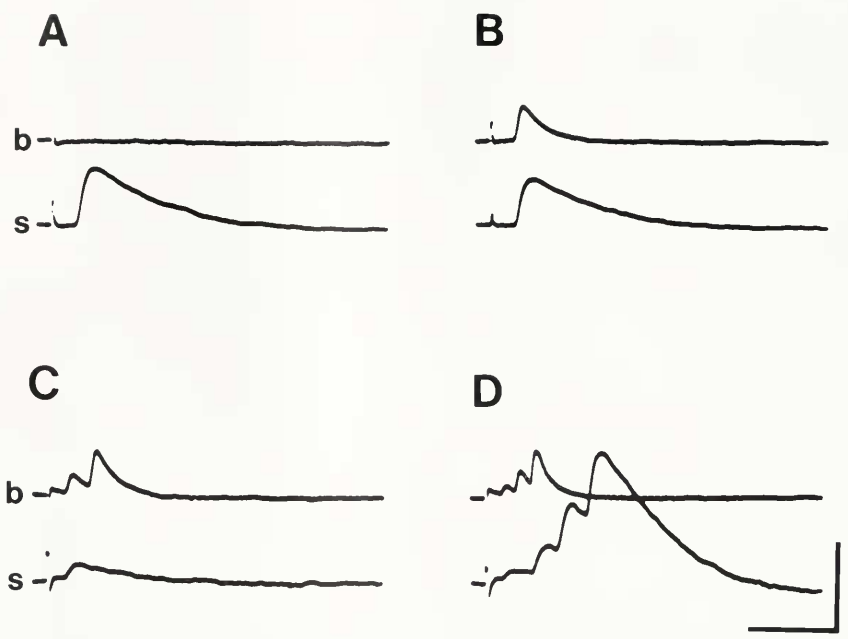

FIGURE 6. The different temperature thresholds for peripheral spike generation in excitor axons to the stretcher (s) and bender (b) muscles in an autotomized walking leg of *Pachygrapsus crassipes*. A. Low intensity stimulus shocks applied to the limb nerve evoked activity in the excitor to the stretcher and an ejp. B. Increasing stimulus intensity recruited the E2 axon to the bender muscle and evoked an ejp. C. At 28.5°C two ejp's were recorded from the bender muscle. D. At 30°C, three ejp's were recorded from the bender muscle and four ejp's from the stretcher muscle. Calibration 10 mV and 10 ms (from Lazurus *et al.*, 1982).

on axons is confined by size constraints, although this technique has been used on unidentified crustacean walking leg axons (Connor, 1975). In such a study, the ionic currents that are involved in the axon spike as well as the depolarizing after-potential could be defined.

One area that has received little attention to date involves the effects of temperature on inhibitory nerves. It is known that temperature increases result in inhibitory synapses failing earlier than excitatory (Fig. 4D; Stephens and Atwood, 1981; White, 1983). However, little is known about the effect of temperature on post- *versus* pre-synaptic inhibition, and the relative temperature-sensitivity of different inhibitors to the same muscle, for example the common inhibitor and specific inhibitors to certain crustacean limb muscles.

Studies on the effect of temperature on the firing patterns of neurons has generally been confined to sensory and motor neurons. Is it possible that temperature also influences neurons that secrete hormones, for example those that have a modulatory effect on neuromuscular transmission (Hoyle *et al.*, 1974; Jacobs and Atwood, 1981b)? This may have a profound effect on the behavior of the intact animal, more than would be predicted by examination of nerve-muscle preparations in autotomized limbs.

Finally, the effects of temperature on interneurons has been given only minimal attention, probably due to difficulties encountered when trying to routinely record from these neurons. Are the effects similar to those observed in the periphery, and,

perhaps equally important, what are the effects of temperature on the properties of electrical synapses? These and many other questions must be answered if the effects of temperature on the behavior of cold-blooded animals are to be fully understood.

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LITERATURE CITED

ARECHIGA, H., AND J. CERBON. 1981. The influence of temperature and deuterium oxide on the spontaneous activity of crayfish motoneurons. *Comp. Biochem. Physiol.* **69**A: 631–636.

ATWOOD, H. L. 1967. Variation in physiological properties of crustacean motor synapses. *Nature* **215:** 57–58.

ATWOOD, H. L., AND G. D. BITTNER. 1971. Matching of excitatory and inhibitory inputs of crustacean muscle fibers. *J. Neurophysiol.* **34:** 157–170.

CHAPELLE, S. 1977. Lipid composition of tissues of marine crustaceans. *Biochem. Syst. Ecol.* **5:** 241–248.

CHAPELLE, S. 1978. The influence of acclimation temperature on the fatty acid composition of aquatic Crustacea (*Carcinus maenas*). *J. Exp. Zool.* **204:** 337–346.

CHAPELLE, S., R. MEISTER, G. BRICHON, AND G. ZWINGELSTEIN. 1979. Influence of temperature on the phospholipid metabolism of various tissues from the crab *Carcinus maenas*. *Comp. Biochem. Physiol.* **58:** 413–417.

CHARLTON, M. P., AND H. L. ATWOOD. 1979. Synaptic transmission: temperature-sensitivity of calcium entry in presynaptic terminals. *Brain Res.* **170:** 543–546.

COLTON, C. K., AND A. R. FREEMAN. 1975. Dual response of lobster muscle fibers to L-glutamate. *Comp. Biochem. Physiol.* **51**C: 275–284.

CONNOR, J. A. 1975. Neural repetitive firing: a comparative study of membrane properties of crustacean walking legs. *J. Neurophysiol.* **38:** 922–932.

CRAWSHAW, L. I. 1983. Effects of thermal acclimation and starvation on temperature selection and activity in the crayfish, *Orconectes immunis*. *Comp. Biochem. Physiol.* **74**A: 475–477.

DALTON, J. C., AND D. E. HENDRIX. 1962. Effects of temperature on membrane potentials of lobster giant axon. *Am. J. Physiol.* **202**(3): 491–494.

DIPOLO, R., AND R. LATORRE. 1972. Effects of temperature on membrane potential and ionic fluxes in intact and dialysed barnacle muscle fibers. *J. Physiol. (Lond.)* **225:** 255–273.

DRIEDZIC, W. M., D. P. SELIVONCHICK, AND B. I. ROOTS. 1976. Alk-1-enyl ether-containing lipids of goldfish (*Carassius auratus* L.) brain and temperature acclimation. *Comp. Biochem. Physiol.* **53:** 311–314.

DUDEL, J., AND R. RUEDEL. 1968. Temperature dependence of electromechanical coupling in crayfish muscle fibers. *Pfluegers Archiv.* **301:** 16–30.

FATT, P., AND B. KATZ. 1953. The electrical properties of crustacean muscle fibers. *J. Physiol. (Lond.)* **120:** 171–204.

FISCHBARG, J. 1972. Ionic permeability changes as the basis of the thermal dependence of the resting potential in barnacle muscle fibers. *J. Physiol. (Lond).* **224:** 149–171.

FISCHER, L., AND E. FLOREY. 1981. Temperature effects on neuromuscular transmission (opener muscle of crayfish, *Astacus leptodactylus*). *J. Exp. Biol.* **94:** 251–268.

FLOREY, E., AND G. HOYLE. 1976. The effects of temperature on a nerve-muscle system of the Hawaiian ghost crab, *Ocypode ceratophthalma* (Pallas). *J. Comp. Physiol.* **110:** 51–64.

FRIEDLANDER, M. J., N. KOTCHABHAKDI, AND C. L. PROSSER. 1976. Effects of cold and heat on behavior and cerebellar function in goldfish. *J. Comp. Physiol.* **112:** 19–45.

GANETZKY, B., AND C-F. WU. 1982. *Drosophila* mutants with opposing effects on nerve excitability: genetic and spatial interactions in repetitive firing. *J. Neurophysiol.* **47:** 501–514.

HARRI, M., AND E. FLOREY. 1977. The effects of temperature on a neuromuscular system of the crayfish, *Astacus leptodactylus*. *J. Comp. Physiol.* **17:** 47–61.

HARRI, M., AND E. FLOREY. 1979. The effect of acclimation temperature on a neuromuscular system of the crayfish, *Astacus leptodactylus*. *J. Exp. Biol.* **78:** 281–293.

HATT, H. 1983. Effect of temperature on the activity of the amino acid receptors in the crayfish walking leg. *J. Comp. Physiol.* **152:** 405–409.

HATT, H., AND D. O. SMITH. 1975. Axon conduction block: differential channeling of nerve impulses in the crayfish. *Brain Res.* **87:** 85–88.

HOYLE, G., D. MOBERLY, AND W. COLQUHOUN. 1974. Dorsal unpaired median insect neurons make neurosecretory endings on skeletal muscle. *J. Exp. Zool.* **187:** 159–165.

HUBBARD, J. I., S. F. JONES, AND E. M. LANDAU. 1971. The effect of temperature change upon transmitter release, facilitation and post-tetanic potentiation. *J. Physiol. (Lond.)* **216:** 591–640.

JACOBS, J. R., AND H. L. ATWOOD. 1981a. Effects of thermal history on long term neuromuscular facilitation in intact crayfish and isolated claw preparations. *J. Comp. Physiol.* **143:** 53–60.

JACOBS, J. R., AND H. L. ATWOOD. 1981b. Long-term facilitation of tension in crustacean muscle and its modulation by temperature, activity and circulating amines. *J. Comp. Physiol.* **144:** 335–343.

JAHROMI, S. S., AND H. L. ATWOOD. 1974. Three-dimensional ultrastructure of the crayfish neuromuscular apparatus. *J. Cell. Biol.* **63:** 599–613.

JENSEN, D. W. 1972. The effect of temperature on transmission of the neuromuscular junction of the sartorius muscle of *Rana pipiens*. *Comp. Biochem. Physiol.* **41**A: 685–695.

KAWAI, N., AND A. NIWA. 1980. Neuromuscular transmission without sodium activation of the presynaptic nerve terminal in the lobster. *J. Physiol. (Lond.)* **305:** 73–85.

KITA, H., K. NARITA, AND W. VAN DER KLOOT. 1980. Effects of temperature on the decline of miniature end-plate potential frequency following a tetanus. *Brain Res.* **190:** 435–445.

KIVIVUORI, L. 1980. Effects of temperature and temperature acclimation on the motor and neural functions in the crayfish *Astacus astacus* L. *Comp. Biochem. Physiol.* **65**A: 297–304.

KIVIVUORI, L. 1983. Temperature acclimation of walking in the crayfish *Astacus astacus*. *Comp. Biochem. Physiol.* **75**A: 513–515.

LANG, F., AND C. K. GOVIND. 1977. Blocking of impulses in specialized regions of crustacean motor axons. *Can. J. Zool.* **55:** 855–861.

LANGLEY, C. K. 1979. Thermal acclimation of a central neurone of *Helix aspersa*. II. Electrophysiological recordings. *J. Exp. Biol.* **78:** 187–200.

LAZURUS, R. E., P. J. STEPHENS, AND N. MINDREBO. 1982. The peripheral generation of action potentials in excitatory motor neurons of a crab. *J. Exp. Zool.* **222:** 129–136.

LEWIS, D. V. 1984. Spike aftercurrents in R15 of *Aplysia:* their relationship to slow inward current and calcium influx. *J. Neurophysiol.* **51:** 387–403.

MARTIN, A. R. 1955. A further study of the statistical composition of the end-plate potential. *J. Physiol. (Lond.)* **130:** 114–122.

MATHESON, D. F., R. OEI, AND B. I. ROOTS. 1980. Changes in fatty acid composition of phospholipids in the optic tectum and optic nerve of temperature-acclimated goldfish. *Physiol. Zool.* **53:** 57–69.

NELSON, D. O., AND C. L. PROSSER. 1981. Temperature-sensitive neurons in the preoptic region of sunfish. *Am. J. Physiol.* **241:** R259–R263.

ORKAND, R. K. 1962. The relation between membrane potential and contraction in single crayfish muscle fibers. *J. Physiol. (Lond.)* **167:** 143–159.

OTSUKA, M., L. L. IVERSON, Z. W. HALL, AND E. A. KRAVITZ. 1966. Release of gamma-aminobutyric acid from inhibitory nerves of lobster. *Proc. Natl. Acad. Sci.* **56:** 1110–1115.

PRIOR, D. J., AND D. S. GREGA. 1982. Effects of temperature on the endogenous activity and synaptic interactions of the salivary burster neurons in the terrestrial slug *Limax maximus*. *J. Exp. Biol.* **98:** 415–428.

PROSSER, C. L., AND E. FAHRI. 1965. Effects of temperature on conditioned reflexes and on nerve conduction in fish. *Z. Vergl. Physiol.* **50:** 92–101.

PROSSER, C. L., AND T. NAGAI. 1968. Effects of low temperature on conditioning in goldfish. Pp. 171–180 in *The Central Nervous System and Fish Behavior*, D. Ingle, ed. University of Chicago Press, Chicago.

ROOTS, B. I., AND P. V. JOHNSTON. 1968. Plasmalogens of the nervous tissue and environment temperature. *Comp. Biochem. Physiol.* **26:** 553–568.

STEPHENS, P. J. 1985. Temperature effects on a slow crustacean system. *Comp. Biochem. Physiol.* (In press.)

STEPHENS, P. J., AND H. L. ATWOOD. 1981. Peripheral generation and modulation of crustacean motor axon activity at high temperatures. *J. Comp. Physiol.* **142:** 309–314.

STEPHENS, P. J., AND R. E. LAZARUS. 1981. Ethanol and temperature modify motor axon firing patterns. *Brain Res.* **226:** 260–263.

STEPHENS, P. J., AND H. L. ATWOOD. 1982. Thermal acclimation in a crustacean neuromuscular system. *J. Exp. Biol.* **98:** 39–47.

STEPHENS, P. J., AND H. L. ATWOOD. 1983. Conversion of synaptic performance in crab motor axons by temperature changes. *J. Comp. Physiol.* **153:** 455–466.

STEPHENS, P. J., P. A. FRASCELLA, AND N. MINDREBO. 1983. Effects of ethanol and temperature on a crab motor axon action potential: a possible mechanism for peripheral spike generation. *J. Exp. Biol.* **103:** 289–301.

TAKEUCHI, A., AND N. TAKEUCHI. 1969. A study of the action of picrotoxin on the inhibitory neuromuscular junction of crayfish. *J. Physiol. (Lond.)* **205:** 377–391.

TAYLOR, R. C. 1984. Thermal preference and temporal distribution in three crayfish species. *Comp. Biochem. Physiol.* **77A:** 513–517.

WARD, D., N. J. CROWLEY, AND T. R. JOHNS. 1972. Effects of temperature at the neuromuscular junction. *Am. J. Physiol.* **222:** 216–219.

WHITE, R. L. 1976. Effects of temperature and low calcium on neuromuscular transmission in the frog. *J. Thermobiol.* **1:** 227–236.

WHITE, R. L. 1983. Effects of acute temperature change and acclimation temperature on neuromuscular function and lethality in crayfish. *Physiol. Zool.* **56:** 174–194.

WIERSMA, C. A. G., AND R. T. ADAMS. 1949. The influence of nerve impulse sequence on the contraction of different crustacean muscles. *Comp. Oecol.* **2:** 20–33.

WIERSMA, C. A. G., AND S. H. RIPLEY. 1952. Innervation patterns of crustacean limbs. *Comp. Oecol.* **2:** 391–405.

YAMAGISHI, S., AND H. GRUNDFEST. 1971. Contributions of various ions to the resting and action potential of crayfish medial giant axons. *J. Membr. Biol.* **5:** 345–365.